Diversity of the basic defect of homozygous *CFTR* mutation genotypes in humans

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Received 31 July 2007
Revised 18 September 2007
Accepted 24 September 2007

ABSTRACT

Background: Knowledge of how *CFTR* mutations other than F508del translate into the basic defect in cystic fibrosis (CF) is scarce due to the low incidence of homozygous index cases.

Methods: 17 individuals who are homozygous for deletions, missense, stop or splice site mutations in the *CFTR* gene were investigated for clinical symptoms of CF and assessed in CFTR function by sweat test, nasal potential difference and intestinal current measurement.

Results: CFTR activity in sweat gland, upper airways and distal intestine was normal for homozygous carriers of G314E or L997F and in the range of F508del homozygotes for homozygous carriers of E92K, W1098L, R553X, R1162X, CFTRdele2(ins186) or CFTRdele2,3(21 kb). Homozygotes for M1101K, 1898+3 A-G or 3849+10 kb C-T were not consistent CF or non-CF in the three bioassays. 14 individuals exhibited some chloride conductance in the airways and/or in the intestine which was identified by the differential response to cAMP and DIDS as being caused by CFTR or at least two other chloride conductances.

Discussion: *CFTR* mutations may lead to unusual electrophysiological or clinical manifestations. In vivo and ex vivo functional assessment of CFTR function and in-depth clinical examination of the index cases are indicated to classify yet uncharacterised *CFTR* mutations as either disease-causing lesions, risk factors, modifiers or neutral variants.

Cystic fibrosis (CF) is an autosomal recessive disorder of all exocrine glands and is caused by mutations in the *Cystic Fibrosis Transmembrane Conductance Regulator* (*CFTR*) gene.[1] CFTR is localised in the apical membrane of epithelial cells and confers cAMP-activatable transport of chloride, bicarbonate and glutathione.[1] The basic defect in CF impairs the apical permeability for chloride and is assessed in humans by the increased chloride concentrations in sweat,[2] the low chloride conductance of upper airway epithelium[3] or the lower chloride secretory response of the intestinal epithelium to secretagogues.[4]

The major mutation F508del is found on about 70% of CF alleles, but all other (>1300) known disease-causing lesions are rare with a frequency ranging from 2% to less than 0.01% in Caucasian populations (http://www.genet.sickkids.on.ca/cftr/).[5] Hence the CFTR phenotype of most mutations other than F508del has been investigated in heterologous expression systems[6] rather than by in vivo or ex vivo analysis of patients' material, because almost all patients with CF are either homozygous for F508del or compound heterozygous for two different *CFTR* mutations. The investigation of compound heterozygous individuals does not provide unequivocal information about the phenotype of the individual mutation and consequently the in vivo phenotype of a *CFTR* mutation can best be studied in homozygotes for a particular *CFTR* mutation.

This report describes the basic defect of individuals who are homozygous for non-F508del *CFTR* mutations. These rare index cases were identified during the last 15 years by *CFTR* mutation analysis of large patient populations in Germany, Italy and Israel. A cohort of F508del homozygous sibpairs from nine European countries[7] was used as reference group. The patients' defective chloride transport across the apical membrane of epithelial cells was measured by the physiological assays employed when diagnosing CF—that is, the sweat test,[2] the nasal potential difference (NPD)[3] and intestinal current measurements (ICM) of freshly excised rectal suction biopsies.[4 7] CFTR function was not detectable in individuals who harbour two *CFTR* null alleles and close to normal in two individuals who are homozygous for non-conservative amino acid substitutions.

SUBJECTS AND METHODS

Subjects

The index cases (table 1) selected from CF centres in Germany, Italy and Israel were investigated at the CF centres in Verona (patients 3 and 4) or Hannover (all other patients). Informed consent was obtained from all patients and at least one parental guide in case of minors. This study was approved by the ethics committee of the Medizinische Hochschule Hannover (study no. 1226). All subjects except patient 2 were offspring of consanguineous marriages which was documented by the parental status of first or second generation cousins in the family tree (patients 1, 3–14) or by the overrepresentation of homozygous marker genotypes (patients 15–17). Before the date of the investigation of the basic defect by NPD and/or ICM, all index cases had been diagnosed with CF. The diagnosis had been based on clinical symptoms compatible with CF and/or a positive family anamnesis, chloride concentrations in the sweat test >40 mval/l and/or two *CFTR* sequence variations not yet described in healthy control populations. The standardised clinical examination at the day of assessment[8] included family anamnesis, patient's history, acute clinical symptoms

and medication, a physical examination, documentation of meconium ileus, meconium ileus equivalent, hepatobiliary disease, diabetes mellitus, fertility, other diseases, anthropometry, determination of pancreatic elastase in stool as an index for the exocrine pancreatic status, a lung function test (the forced vital capacity and the forced expiratory volume in 1 s were determined by spirometry and expressed as a predictive value based on Knudson's formulas[9]), bacteriology of sputum or deep throat swab, serum IgG and IgE and antipseudomonal IgG. After the clinical examination a Gibson–Cooke pilocarpine iontophoresis sweat test,[2] NPD[3] and ICM[4] measurements were performed within the next 72 h.

Mutation analysis at the *CFTR* locus

A cascade approach was taken to identify *CFTR* mutations in genomic DNA from nuclear blood cells.[10 11] K-EDTA blood samples were taken from the patient and, at the Hannover site, also from the parents. First, frequent *CFTR* mutations were directly tested by established polymerase chain reaction (PCR)-based protocols guided by the parental *CFTR* haplotypes of the index case. Next, the 27 exons of the *CFTR* gene together with their flanking intron regions were amplified separately by PCR. Before 1995, sequence variations were sought by single strand conformation polymorphism (SSCP) or restriction enzyme based analysis of PCR product. All non-F508del mutations detected by direct testing or anomalous migration behaviour in SSCP were confirmed by direct genomic sequencing of the respective exon. Starting by 1995, SSCP was omitted and the whole coding and flanking intron sequences were determined by direct genomic sequencing as the second step. Finally, specimens which were refractory to amplification by PCR were scanned for genomic rearrangements by Southern hybridisation with PCR generated genomic probes encompassing the respective exon and flanking intron sequences. By this cascade approach a 100% detection rate of two *CFTR* mutations had been achieved in a cohort of 330 exocrine pancreas insufficient German patients with CF. Of 52 exocrine pancreas sufficient subjects with CF, two *CFTR* mutations were detected in 42 patients and only one mutation in 10 patients.

Transepithelial NPD measurements[3 7]

Access to the subcutaneous space was obtained by a needle filled with NaCl solution inserted subcutaneously into the forearm. The lower nasal turbinate was superfused with a polyethylene tube (PE-50) connected via syringes to the various superfusing solutions. Both the needle and the PE-50 tubing were connected to a high impedance voltmeter via Ag/AgCl electrodes and agar/saline-filled salt bridges. The basal PD was measured by superfusing NaCl buffer A (120 mM NaCl, 25 mM NaGluconate, 0.4 mM NaH_2PO_4, 2.4 mM Na_2HPO_4) over the nasal mucosa. Subsequently, electrogenic sodium absorption was blocked with 0.1 mM amiloride in buffer A. To determine the basal chloride conductance, the nasal mucosa was superfused with buffer B (145 mM NaGluconate, 0.4 mM NaH_2PO_4, 2.4 mM Na_2HPO_4, 0.1 mM amiloride). Finally the responsiveness to 0.1 mM isoproterenol in buffer B, which typically activates CFTR chloride channels, was tested.

Intestinal current measurements

ICM was performed on freshly obtained rectal suction biopsies in a micro-Ussing chamber according to our published protocol.[4 7] The biopsies equilibrated in Meyler's buffer (gassed with 95% O_2–5% CO_2)[4] were sequentially exposed to the mucosal (*M*) and/or serosal (*S*) side to: (1) amiloride (10^{-4} M, *M*); (2) indomethacin (10^{-5} M, *M*+*S*), to reduce basal chloride secretion by inhibiting the endogenous prostaglandin formation; (3) carbachol (10^{-4} M, *S*), to initiate the cholinergic Ca^{2+}-linked chloride secretion; (4) forskolin (10^{-5} M, *M*+*S*) together with 8-bromo-cAMP (10^{-3} M, *M*+*S*), to open cAMP-dependent Cl^- channels like CFTR; (5) 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid (DIDS, 2.10^{-4} M, *M*), to inhibit DIDS-sensitive Cl^- transporters like the Ca^{2+}-dependent Cl^- channels and the ORCC; (6) histamine (5.10^{-4} M, *S*), to reactivate the Ca^{2+}-dependent chloride secretory pathway.[4]

RESULTS

Seventeen individuals homozygous for a non-F508del *CFTR* mutation were recruited for the study. The patients' clinical characteristics and the sweat test, NPD and ICM values are shown in tables 1–3. The electrophysiological phenotype of the patients' basic defect (table 4) was compared with that of F508del homozygous CF siblings who had been studied previously by the same protocol and the same core team of investigators.[7] All F508del homozygotes had CF-typical sweat test, NPD and ICM values (tables 2 and 3).[7]

Expression of functional CFTR was expected to be absent in patients harbouring two *CFTR* null alleles. The homozygotes for the large out-of-frame 21 kb deletion of exons 2 and 3[12] of the *CFTR* gene (Fig 1B) or the nonsense mutation R553X showed indeed full-blown CFTR loss-of-function phenotypes in the bioassays. The two R1162X homozygous patients, however, had some chloride secretory activity in their intestine that consisted of both DIDS-sensitive and DIDS-insensitive components (fig 1D, tables 4 and 5).

Residual chloride conductance was detected in subjects with splice site mutations that permit production of some wild type CFTR. The mutation 3849+10 kb C-T is known to activate a cryptic splice acceptor site in intron 19 and leads to variable levels of both aberrant and correct CFTR mRNA transcripts modulated by splicing factors in a tissue dependent manner.[13 14] Accordingly we measured sweat chloride concentrations and basal NPD values in both the normal and the CF range in two 3849+10 kb C-T homozygous siblings, whereas in contrast only minute chloride secretion like in most F508del homozygotes was detected in the ICM. The donor splice mutation 1898+3 A-G in intron 12 of *CFTR*[15] affects a not obligatorily conserved position. The homozygous index case presented an electrophysiological phenotype intermediate between CF and non-CF (tables 1–3). The DIDS-insensitive chloride secretory responses to cAMP, carbachol and histamine in the ICM were higher than those ever seen in patients with an established CF diagnosis, but were lower than measured before in all non-CF controls (fig 1B). Consistent with this intermediate phenotype of the basic defect, the currently 15-year-old patient developed only minimal symptoms of CF respiratory and gastrointestinal disease and required virtually no treatment.

The in-frame deletion of exon 2 detected by the authors within the frame of this study showed a variable manifestation of the basic defect with residual cAMP-activatable DIDS-sensitive or DIDS-insensitive chloride secretion in two of the three patients (tables 4 and 5). Absence or presence of residual chloride secretory activity in the intestine was associated with severe or very mild courses of clinical CF disease in the three subjects who received no adequate symptomatic therapy of CF for prolonged periods of time. The ICM data suggest that the CFTR mutant lacking exon 2 may confer some residual chloride secretory activity to the intestine.

Table 1 Patients' characteristics

Patient number	*CFTR* genotype	Sex	Age at diagnosis	Symptoms at diagnosis	Pancreatic status	Meconium ileus	Colonisation with *Pseudomonas aeruginosa*	Age at onset of *P aeruginosa* colonisation	Peculiar features
Out-of-frame deletion									
1	CFTRdele2,3(21 kb)/CFTRdele2,3(21 kb)	F	Birth	Meconium ileus	PI	Yes	Yes	6 y 6 mo	Diabetes mellitus
Nonsense mutation									
2	R553X/R553X	F	16 mo	Steatorrhea, failure to thrive	PI	No	Yes	10 y	
3	R1162X/R1162X	F	5 mo	Malnutrition	PI	No	Yes	18 y 4 mo	Diabetes mellitus, atopy
4	R1162X/R1162X	M	18 d	Positive family anamnesis	PI	No	No		Atopy
Splice site mutation									
5	1898+3 A-G/1898+3 A-G	F	3 mo	Pneumonia	PS	No	No		Repetitive episodes of salt loss and dehydration
6	3849+10 kb C-T/3849+10 kb C-T	M	20 y 5 mo	Pneumonia, dyspnoea, hypoxaemia	PS	No	Yes	26 y 6 mo	Lung transplant, splenomegaly
7	3849+10 kb C-T/3849+10 kb C-T	M	11 y 2 mo	Bronchitis, positive family anamnesis	PS	No	Yes	12 y 3 mo	
In-frame deletion									
8	CFTRdele2(ins186)/CFTRdele2(ins186)	M	3 mo	Dehydration	PI	No	No		
9	CFTRdele2(ins186)/CFTRdele2(ins186)	F	Birth	Meconium ileus	PI	Yes	Yes	9 y	lung transplant
10	CFTRdele2(ins186)/CFTRdele2(ins186)	M	2 y	Bronchitis	PI	No	Yes	4 y	
Missense mutation									
11	E92K/E92K	M	8 mo	Salt loss, muscle weakness	PS	No	Yes	11 y 9 mo	Fatigue, muscle weakness and salt loss during exercise
12	G314E/G314E	F		Bronchitis	PS	No	No		
13	L997F/L997F	F			PS	No	No		Family anamnesis suspicious for CF, prenatal analysis of *CFTR*
14	W1098L/W1098L	M	2 mo	Salt loss, failure to thrive	PS	No	No		
15	M1101K/M1101K	F	5y 9 mo	Pneumonia	PI	No	No		
16	M1101K/M1101K	F	4 y 6 mo	Airways infections	PS	No	No		
17	M1101K/M1101K	M	1 mo	Positive family anamnesis	PI	No	No		

d, days; F, female, M, male; mo, months; PI, exocrine pancreatic insufficient; PS, exocrine pancreatic sufficient; y, years
Sibpairs: patients 3 & 4, 6 & 7, 9 & 10, 15, 16 & 17.

Table 2 Assessment of basic defect (A): sweat tests and nasal potential difference (NPD) measurements (mV)

Patient number	*CFTR* genotype	Sweat chloride concentration (mval/l)		Basal PD (mV)	Change in PD (mV)	
		Day of assessment	Prior tests (age)		Amiloride	Chloride-free + isoproterenol
Out-of-frame deletion						
1	CFTRdele2,3(21 kb)/CFTRdele2,3(21 kb)	103	95 (10 mo)	−60	22	−10
Nonsense mutation						
2	R553X/R553X	96	100 (16 mo)	−62	34	−7
3	R1162X/R1162X	98	110 (2 y 1 mo)	−48	23	−4
4	R1162X/R1162X	104	112 (1 mo)	−39	30	0
Splice-site mutation						
5	1898+3 A-G/1898+3 A-G	73	69 (4 mo)	−33	21	−3
6	3849+10 kb C-T/3849+10 kb C-T	92	64 (20 y 5 mo) 49 (28 y 4 mo)	−44	30	−12
7	3849+10 kb C-T/3849+10 kb C-T	20	50 (11 y 2 mo)	−27	12	+3
In-frame deletion						
8	CFTRdele2(ins186)/CFTRdele2(ins186)	102	134 (4 mo)	−45	30	−1
9	CFTRdele2(ins186)/CFTRdele2(ins186)	100	119 (9 y)	−48	31	−8
10	CFTRdele2(ins186)/CFTRdele2(ins186)	131	100 (4 y)	−58	41	−12
Missense mutation						
11	E92K/E92K	118	93 (8 mo)	−52	20	−11
12	G314E/G314E	15	43 (6 y 2 mo)	−19	4	−16
13	L997F/L997F	8				
14	W1098L/W1098L	107	118 (2 mo)			
15	M1101K/M1101K	108	120	−56	33	−16
16	M1101K/M1101K	130	120	−64	26	−15
17	M1101K/M1101K	118		−29	13	−10
	F508del/F508del (n = 74)[7]	106±22		−56±10	28±9	−8±5
	non-CF (n = 25)	16±9		−20±10	11±6	−30±8

Sibpairs: patients 3 & 4, 6 & 7, 9 & 10, 15, 16 & 17.

Seven study participants were homozygous for a missense mutation in the *CFTR* gene (table 4). By the time of writing, none of the 5- to 23-year-old individuals had yet experienced any severe exacerbation of CF pulmonary or gastrointestinal disease. The homozygotes for E92K,[16] W1098L or M1101K[17] showed highly elevated sweat chloride concentrations in the CF range on several occasions, whereas the homozygotes for G314E[18] or L997F[19 20] had normal sweat electrolytes like non-CF healthy controls (table 2). The NPD responses were heterogeneous in the group. Only the E92K homozygote showed the CF pattern of a large response to amiloride and of a low chloride diffusion potential. The missense mutation E92K results from a G-to-A transition in the first base of exon 4 and hence may not also lead to the substitution of a glutamate by a lysine but also may affect splicing as it has been observed for the stop mutation E92X.[21] The G314E and the M1101K homozygotes exhibited an intermediate chloride secretory phenotype between typical CF and typical non-CF. Rectal epithelial tissue from all seven subjects exhibited substantial DIDS-insensitive chloride secretory responses indicating that all analysed CFTR mutants can transport chloride. The transport rates were in the upper CF range (E92K, W1098L, one M1101K sibling), in the intermediate range between CF and non-CF (the other two M1101K siblings) or in the normal range (L997F, G314E) (fig 1C). The tissue specimens from two M1101K homozygous siblings expressed two patterns of chloride secretory responses that are consistent with the presence of both CFTR and the alternative chloride channel ORCC (fig 1E, table 5).[7] Since the outcome of NPD, ICM, sweat test and clinical examination was normal in the G314E or L997F homozygotes, the diagnosis of CF that had been based on mutation reports in the literature,[18 19] positive family anamnesis or suggestive respiratory symptoms, was withdrawn for these two individuals.

DISCUSSION

This first study of how non-F508del homozygous *CFTR* genotypes translate into the basic defect in the major affected organs in vivo, uncovered the full range from complete loss-of-function to normal non-CF phenotypes. All patients except patient 2 descended from consanguineous marriages characterised by an overrepresentation of homozygous genotypes. Hence we expected the sibpairs in our cohort to be concordant in their manifestations of aberrant epithelial chloride conductance, but this was not the case. The siblings showed an individual rather than a shared electrophysiological signature in NPD and ICM (tables 2 and 3), suggesting that individual factors modify the CF phenotype at the level of the basic defect even though it is more closely related to the mutation genotype than any clinical symptom.

More than 90% of CF patients are homozygous or compound heterozygous for F508del,[1] and consequently our perception of CF disease is dominated by this major mutation. Loss-of-function mutations such as CFTRdele2,3(21 kb) or R553X were not distinguishable in their clinical phenotypes from those of F508del homozygotes, although the consequences at the molecular level such as nonsense mediated decay and exon skipping[22] or defective maturation and trafficking of protein[1] are different.

All other *CFTR* mutations investigated in this study translated into facets of disease and basic defect that are atypical for the most common genotype. Splice site mutations, for example, were associated with progressive lung disease and a

Table 3 Assessment of basic defect (B): intestinal current measurements (ICM)

			Response to		Interpretation		
Patient number	***CFTR genotype***	**Carbachol (% reverse signal)**	**cAMP**	**DIDS + histamine (% rev. signal)**	**Residual chloride conductance**	**Sensitivity to DIDS**	**cAMP response**
Out-of-frame deletion							
1	CFTRdele2,3(21 kb)/CFTRdele2,3(21 kb)	100	None	100	No	No	No
Nonsense mutation							
2	R553X/R553X	100	None	100	No	No	No
3	R1162X/R1162X	0	Yes	40	Yes	Yes	Yes
4	R1162X/R1162X	45	Yes	90	Yes	Yes	Yes
Splice-site mutation							
5	1898+3 A-G/1898+3 A-G	0	Intermediate	0	Yes	No	Yes
6	3849+10 kb C-T/3849+10 kb C-T	70	Minute	70	Yes	No	Yes
7	3849+10 kb C-T/3849+10 kb C-T	100	Minute	100	Minute	No	Yes
In-frame deletion							
8	CFTRdele2(ins186)/CFTRdele2(ins186)	100	Small	100	Small	Yes†	Yes
9	CFTRdele2(ins186)/CFTRdele2(ins186)	100	None	100	No	No	No
10	CFTRdele2(ins186)/CFTRdele2(ins186)	40	Small	40	Yes	No	Yes
Missense mutation							
11	E92K/E92K	40	Small	50	Yes	No	Yes
12	G314E/G314E	0	Intermediate	0	Yes	No	Yes
13	L997F/L997F	0	Normal	0	Yes	No	Yes
14	W1098L/W1098L	40	Small	40	Yes	No	Yes
15	M1101K/M1101K	30	Intermediate	70	Yes	Yes	Yes
16	M1101K/M1101K	0	Intermediate	30	Yes	Yes	Yes
17	M1101K/M1101K	50	Small	100	Yes	Yes	Yes
Non-CF*		0	Normal	0	Yes	No	Yes
		>20 μA/cm²	>20 μA/cm²	>20 μA/cm²			
CF*		0–100	None/small	0–100	Yes or no	Yes or no	Yes or no
(All patients)		<10 μA/cm²	<5 μA/cm²	<10 μA/cm²			
Subgroups:							
CF*		90–100	None	90–100	No	No	No
(no residual chloride secretion)		<0 μA/cm²	<1 μA/cm²	<0 μA/cm²			
CF*		0–90	Small	0–90	Yes	No	Yes
(residual chloride secretion)		0–10 μA/cm²	<5 μA/cm²	0–10 μA/cm²			
CF*		0–100	None/small	100	Yes	Yes	Yes or no
(alternative chloride secretion)		<10 μA/cm²	<5 μA/cm²	<0 μA/cm²			

*Range and threshold of values for the different categories are based on previously published data (summarised in De Jonge *et al*[4]).
†cAMP response sensitive to DIDS in patient 8.

Table 4 Evaluation of the basic defect in individuals with homozygous *CFTR* mutation genotypes

		Values within the range of											
		F508del homozygotes					Intermediate			Non-CF healthy controls			
				NPD‡				NPD‡				NPD‡	
Patient number	CFTR mutation	Sweat chloride*	ICM†	Basal	Amil	Cl^--free	ICM†	Basal	Cl^--free	Sweat chloride*	ICM†	Basal	Amil
Out-of-frame deletion													
1	CFTRdele2,3(21 kb)	X	X	X	X	X							
Nonsense mutation													
2	R553X	X	X	X	X	X							
3	R1162X	X	X	X	X	X							
4	R1162X	X	X		X	X		X					
Splice-site mutation													
5	1898+3 A-G	X			X	X	X	X					
6	3849+10 kb C-T	X	X	X	X	X							
7	3849+10 kb C-T		X			X				X		X	X
In-frame deletion													
8	CFTRdele2(ins186)	X	X	X	X	X							
9	CFTRdele2(ins186)	X	X	X	X	X							
10	CFTRdele2(ins186)	X	X	X	X	X							
Missense mutation													
11	E92K	X	X	X	X	X							
12	G314E								X	X	X	X	X
13	L997F									X	X		
14	W1098L	X	X										
15	M1101K	X		X	X		X		X				
16	M1101K	X		X	X		X		X				
17	M1101K	X	X			X						X	X

Sibpairs: patients 3 & 4, 6 & 7, 9 & 10, 15, 16 & 17.
Consanguineous parents of patients: 1, 3–14
*Sweat chloride concentrations determined in Gibson–Cooke pilocarpine iontophoresis sweat tests.
†DIDS-insensitive cAMP-activatable chloride secretory responses in rectal suction biopsies as surrogate parameter for CFTR function.[4 7]
‡NPD measurements of the basal PD (basal), the response to amiloride (amil) and the cumulative response to chloride-free solution plus isoprotenerol (Cl^- -free).

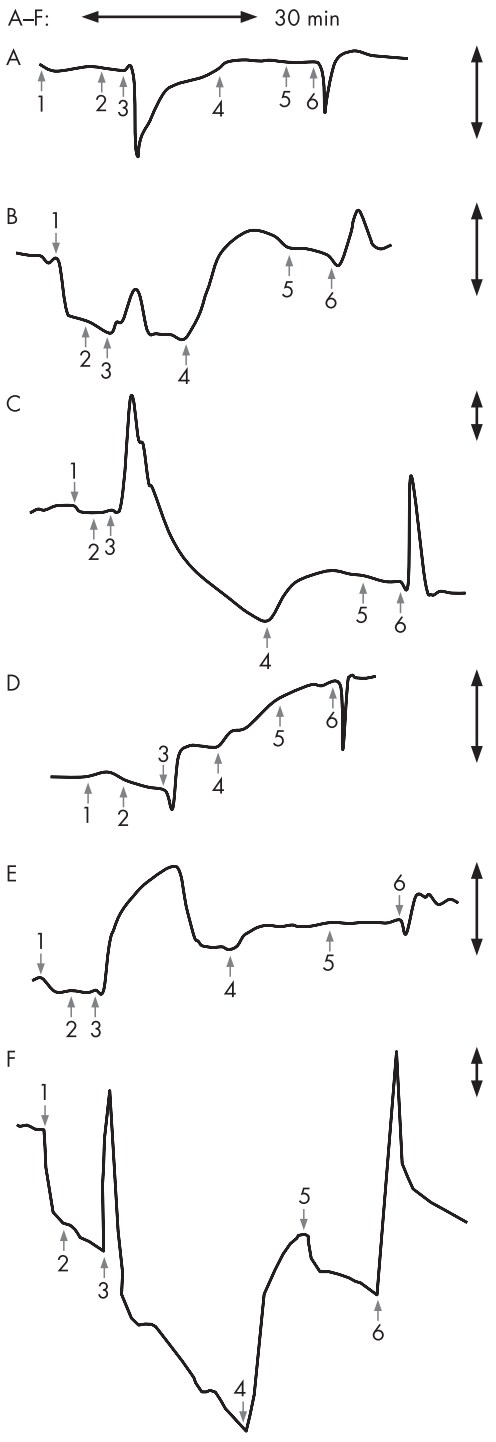

Figure 1 Recordings of short circuit currents. ICM recordings of individuals homozygous for *CFTRdele2,3(21 kb)* (A), *1898+3 A-G* (B), *G314E* (C), *R1162X* (D), *M1101K* (E) or of a healthy non-CF individual (F). The horizontal double-headed arrow corresponds to a recording time of 30 min and each vertical double-headed arrow to a current of 10 μA/cm². Please note the twofold contracted scale for tracings C and F. The arrows indicate the addition of amiloride (1), indomethacin (2), carbachol (3), cAMP/forskolin (4), DIDS (5) and histamine (6). The tracings show no (A), intermediate (B) or normal CFTR activity (C, F). The chloride secretory responses of patients D and E are partially caused by the expression of alternative chloride channels.

normal sweat test in case of 3849+10 kb C-T[13] and with minimal disease and substantial chloride secretion beyond the CF range in the ICM in case of 1898+3 A-G.

Table 5 Patients' patterns of residual chloride secretory responses in intestinal current measurements (ICM)

Chloride secretory response	Sensitivity to		Interpretation	
	cAMP	DIDS	Ion channel	Patient number
Yes	Yes	No	CFTR	3, 4, 5, 6, 7, 10, 11, 12, 13, 14, 15, 16, 17
Yes	Yes	Yes	CACC/ORCC	3, 4, 8, 15, 16, 17
No				1, 2, 9

Missense mutations are a guide to dissect the function of CFTR motifs and domains. E92K and D110H (data not shown) are located in the first ectoplasmic loop. The index cases were prone to severe salt loss upon exposure to heat or exercise, but otherwise are overweight and have normal lung function. Hence, the integrity of the loop at these positions seems to be essential for the reabsorption of salt from the sweat duct, but less relevant for ion secretion in airways and intestine.

The mutations W1098L and M1101K reside in the cytoplasmic loop 4 (residues 1035–1102). Heterologously expressed, recombinant mutants W1098R and M1101K were defective in CFTR maturation and non-functional in anion efflux assays.[6] In contrast, the four homozygous patients unequivocally demonstrated substantial residual chloride conductance in the NPD and ICM bioassays (table 4). In vitro and in vivo CFTR mutant phenotypes do not match. Rescue mechanisms are probably operating in the patients which were absent in the heterologous host cells in vitro. This example makes the point that in vitro findings on recombinant mutants should be interpreted with caution when utilised for counselling of patients and their relatives.

The non-conservative amino acid substitutions L997F and G314E did not impair chloride conductance in sweat glands, airways and intestine. The normal phenotype of the glycine-to-glutamate substitution is particularly striking. The *CFTR* mutation database lists 20 missense mutations with a change of glycine to an acidic amino acid or vice versa, three of which are classified as benign sequence variants and eight of which as disease-causing. Several well characterised severe mutations occur in the evolutionarily conserved Walker (G1244E, G1249E) or dodecapeptide motifs (G551D, G1349D) of the ABC transporter CFTR.[1] The missense mutants G622D[23] in the regulatory domain and G314E in the fifth transmembrane region led to no clinical symptoms of CF. The pathogenetic role of nine mutations is still unresolved. The same amino acid substitution thus affects CFTR function to a variable extent depending on its localisation within the protein. In other words, the molecular pathology of a missense mutant is hard to predict, because CFTR topology, function and processing are complex. If mutation analysis uncovers an uncommon and/or yet uncharacterised missense mutation, diagnostic bioassays and in-depth clinical examination of the index cases need to be pursued in order to classify the clinical impact of an amino acid substitution as either neutral, benign or severe.

Our highly selected study cohort of individuals with rare homozygous *CFTR* genotypes showed an association with mild or asymptomatic clinical manifestation if CFTR function was in the intermediate or normal range in both NPD and ICM. This positive correlation between the amount of residual CFTR function and milder disease did not, however, apply to the individual case, if NPD and/or ICM values were within the CF range. The two 3849+10 kb C-T homozygotes who started to develop symptoms of CF respiratory disease during adolescence,

and were diagnosed at the oldest age in our cohort, showed the most severe progression of disease during adulthood leading to lung transplantation and death; however, patients 1 and 2, with loss-of-function electrophysiological signature, experienced at the same age a comparatively high quality of life with no significant deterioration of their respiratory or gastrointestinal disease.

In summary, while our study could clearly distinguish individuals with asymptomatic or atypical CF, no prognostic value could be derived for the individual case from the amount of residual CFTR-mediated chloride secretion if the results were within the CF range. This conclusion should be borne in mind during genetic counselling of patients or parental guides that typically takes place early in the life of a CF patient.

Acknowledgements: We would like to thank patients and their parental guides for the participation in the study and our colleagues at the CF clinics in Bonn, Düsseldorf, Esslingen, Frankfurt, Gießen, Göttingen, München, Münster, Tel Aviv and Verona for organizational help. The study was supported by a grant from the Deutsche Forschungsgemeinschaft to BT (SFB 621, project C7). The generous support of participating families by the Christiane Herzog Stiftung is gratefully acknowledged.

Competing interests: None.

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Diversity of the basic defect of homozygous *CFTR* mutation genotypes in humans

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J Med Genet 2008 45: 47-54
doi: 10.1136/jmg.2007.053561